

WHAT IS CLAIMED IS:

1. A method for determining the presence or absence of a nucleic acid hybrid in a sample comprising the following steps:

(a) providing a reaction mixture comprising (i) a sample that may contain a nucleic acid hybrid that comprises a 3'-terminus, (ii) pyrophosphate, (iii) an enzyme that catalyzes the release of a nucleotide from a nucleic acid hybrid, by pyrophosphorolysis of the 3'-terminus of a strand of the nucleic acid hybrid in the presence of pyrophosphate, and (iv) a suitable nucleotide that can be incorporated in the place of said released nucleotide;

(b) maintaining said reaction mixture for a time period and under conditions that permit (i) pyrophosphorolysis of the 3'-terminus of a strand of a nucleic acid hybrid to produce a released nucleotide and a modified 3'-terminus as well as (ii) the incorporation of said suitable nucleotide into the modified 3'-terminus of the nucleic acid hybrid to produce an incorporated modified 3'-terminus, thereby forming a treated sample; and

(c) assaying the treated sample to determine whether incorporation of said suitable nucleotide into the hybrid occurred and thereby determining the presence or absence of a nucleic acid hybrid in said sample.

2. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 1 wherein the nucleotide suitable for incorporation

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into the modified 3'-terminus of the nucleic acid hybrid includes a label.

3. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 1 wherein the method of assaying whether incorporation of a suitable nucleotide occurred comprises determining whether the label is associated with the nucleic acid hybrid.

4. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 2 wherein the label is a fluorescent label.

5. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 3 wherein determining whether the fluorescent label is associated with the nucleic acid hybrid comprises the following steps:

(a) providing a treated sample comprising a fluorescence-modulating oligonucleotide that comprises a fluorescence quencher or enhancer that is complementary to the incorporated modified 3'-terminus having the fluorescent label;

(b) maintaining the treated sample under conditions and for a time period sufficient to permit hybridization of said incorporated modified 3'-terminus with said fluorescence-modulating oligonucleotide to form a fluorescence-modulated hybrid;

(c) irradiating the treated sample with radiation that is appropriate for causing fluorescence of said label; and

(d) monitoring the fluorescence of said treated sample comprising a fluorescence-modulating

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oligonucleotide, whereby a difference in fluorescence indicates the presence of a fluorescence-modulated hybrid and therefore said nucleic acid hybrid in the sample.

6. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 1 wherein said nucleic acid hybrid is formed between a target nucleic acid strand and a probe nucleic acid strand.

7. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 2 wherein the label is a capture label.

8. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 1 wherein said nucleic acid hybrid comprises a label.

9. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 8 wherein said label is a capture label.

10. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 2 wherein said nucleotide suitable for incorporation into the modified 3'-terminus of the nucleic acid hybrid is a chain terminating or other polymerization-blocking nucleotide.

11. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 6 wherein assaying to determine whether incorporation of said suitable nucleotide occurred is

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carried out by detecting an increase in the length of said probe.

12. A method for determining the presence or absence of a nucleic acid target in a sample comprising the following steps:

(a) providing a reaction mixture comprising (i) a sample that may contain a nucleic acid target, (ii) a nucleic acid probe corresponding to said nucleic acid target, (iii) pyrophosphate, (iv) an enzyme that catalyzes the release of a nucleotide from a nucleic acid hybrid, which comprises a 3'-terminus, by pyrophosphorolysis of the 3'-terminus of a strand of the nucleic acid hybrid in the presence of pyrophosphate, and (v) a suitable nucleotide that can be incorporated in the place of said released nucleotide;

(b) maintaining said reaction mixture for a time period and under conditions that permit (i) hybridization of the nucleic acid target with the nucleic acid probe to form a nucleic acid hybrid that comprises a 3'-terminus, (ii) pyrophosphorolysis of the 3'-terminus of a strand of a nucleic acid hybrid to produce a released nucleotide and a modified 3'-terminus as well as (iii) the incorporation of said suitable nucleotide into the modified 3'-terminus of the nucleic acid hybrid to produce an incorporated modified 3'-terminus, thereby forming a treated sample; and

(c) assaying the treated sample to determine whether incorporation of said suitable nucleotide occurred and thereby determining the presence or absence of the nucleic acid target in said sample.

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13. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 12 wherein said reaction mixture may comprise a plurality of nucleic acid targets and their corresponding nucleic acid probes.

14. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 13 wherein said nucleic acid probes are distinguishable from one another on the basis of length, thereby permitting the determination of the presence or absence of a plurality of nucleic acid targets.

15. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 13 wherein the suitable nucleotides incorporated to form the incorporated modified 3'-terminus permit distinction between the probes, thereby permitting the determination of the presence or absence of a plurality of nucleic acid targets.

16. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 15 wherein the nucleic acid probes having incorporated modified 3'-termini are distinguishable from one another on the basis of the suitable nucleotide incorporated or on the basis of length, thereby permitting the determination of the presence or absence of a plurality of nucleic acid targets.

17. The method for determining the presence or absence of a nucleic acid target in a

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sample according to claim 12 wherein said reaction mixture may comprise a plurality of nucleic acid targets that differ from one another by a single base.

18. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 17 wherein said plurality of nucleic acid targets differ from one another by a single base at an interrogation position.

19. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 18 wherein the penultimate 3'-terminal residue of the corresponding nucleic acid probe base pairs with the interrogation position of the nucleic acid target.

20. A method for determining the presence or absence of a specific nucleic acid base at an interrogation position of a nucleic acid target in a sample comprising the following steps:

(a) providing a reaction mixture comprising (i) a sample that may contain a nucleic acid target having a nucleic acid residue at an interrogation position, (ii) a nucleic acid probe comprising a nucleic acid residue in its 3'-terminus that base pairs with the interrogation position of the nucleic acid target when the nucleic acid target and the nucleic acid probe are hybridized to form a nucleic acid hybrid, (iii) pyrophosphate, (iii) an enzyme that catalyzes the release of a nucleotide from a nucleic acid hybrid, which comprises a 3'-terminus, by pyrophosphorolysis of the 3'-terminus of a strand of the nucleic acid hybrid in the presence

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of pyrophosphate, and (iv) a suitable nucleotide that can be incorporated in the place of said released nucleotide;

(b) maintaining said reaction mixture for a time period and under conditions that permit (i) formation of a nucleic acid hybrid between the nucleic acid probe and the nucleic acid target, (ii) pyrophosphorolysis of the 3'-terminus of a strand of a nucleic acid hybrid to produce a released nucleotide and a modified 3'-terminus and (iii) the incorporation of said suitable nucleotide into the modified 3'-terminus of the nucleic acid hybrid to produce an incorporated modified 3'-terminus, thereby forming a treated sample; and

(c) assaying the treated sample to determine whether incorporation of said suitable nucleotide occurred and thereby determining the presence or absence of a specific nucleic acid base at an interrogation position of a nucleic acid target in said sample.

21. The method for determining the presence or absence of a specific nucleic acid base at an interrogation position of a nucleic acid target in a sample according to claim 20 wherein the nucleic acid residue of the nucleic acid probe that corresponds with the interrogation position of the nucleic acid target is the 3'-terminal residue.

22. The method for determining the presence or absence of a specific nucleic acid base at an interrogation position of a nucleic acid target in a sample according to claim 20 wherein the nucleic acid residue of the nucleic acid probe that corresponds with the interrogation position of the

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nucleic acid target is the penultimate 3'-terminal residue.

23. A reaction mixture comprising (i) a sample that may contain a nucleic acid hybrid that comprises a 3'-terminus, (ii) pyrophosphate, (iii) an enzyme that catalyzes the release of a nucleotide from a nucleic acid hybrid, which comprises a 3'-terminus, by pyrophosphorolysis of the 3'-terminus of a strand of the nucleic acid hybrid in the presence of pyrophosphate, and (iv) a suitable nucleotide that can be incorporated in the place of said released nucleotide.

24. The method for determining the presence or absence of a nucleic acid hybrid in a sample according to claim 1 wherein said nucleic acid hybrid is affixed to a solid support.

25. The method for determining the presence or absence of a nucleic acid hybrid in a sample according to claim 24 wherein said nucleic acid hybrid is affixed to a solid support through attachment of a strand of the nucleic acid hybrid to said solid support.

26. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 9 wherein said nucleic acid hybrid is attached to a solid support through said capture label and wherein said suitable nucleotide is a different label used for assaying the treated sample to determine whether incorporation of said suitable nucleotide occurred.

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(a) providing a reaction mixture comprising (i) a sample contains a nucleic acid hybrid that comprises a 3'-terminus, (ii) pyrophosphate, (iii) an enzyme that catalyzes the release of a nucleotide from a nucleic acid hybrid, by pyrophosphorolysis of the 3'-terminus of a strand of the nucleic acid hybrid in the presence of pyrophosphate, and (iv) a suitable nucleotide that can be incorporated in the place of said released nucleotide;

(c) assaying the treated sample to determine where incorporation of said suitable nucleotide into the hybrid occurred and thereby determining nucleotide sequence of the nucleic acid hybrid.

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